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Intrageneric comparisons in urine-concentrating capacity and renal morphology among three species of *Akodon* from different geographic rainfall regimens

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Abstract

Urine-concentrating capacity and renal morphology were examined for *Akodon azarae*, *A. iniscatus* and *A. cursor* from different geographic rainfall regimens. *A. azarae* and *A. cursor* rejected sodium chloride solution above 0.45 M, whereas *A. iniscatus* did not. *A. azarae* and *A. iniscatus* may concentrate urine to similar osmolarity values. These values were higher than those observed for *A. cursor*. Percent medullary thickness of the kidney for *Akodon* was related to both geographic rainfall regimens and urine osmolarity.

Key words: *Akodon* species, urine osmolarity, renal morphology

Introduction

Water is the primary constituent of animals. Mammals, particularly those adapted to arid habitats, can minimise their urinary water loss due to the fact that their kidneys can produce urine that is significantly more concentrated than their plasma (Brooker and Withers 1994). In mammals, adaptive radiation into new environments may involve both physiological and structural adaptations of the kidney. Hence, interspecific variation in these characteristics may represent adaptive responses to habitat differences in water availability.

Urine-concentrating ability, and consequently the ability to conserve water have been evaluated in many morphological and functional studies on the mammalian kidney. These data have been correlated, in many instances, with water availability and habitat distribution. One example is given by an earlier study performed by Sperber (1944) after examining species representing most mammalian orders, highlighting the relationship between mammal distribution related to climatic factors and their ability to concentrate urine throughout renal morphology. Later on, Schmidt-Nielsen and O'Dell (1961), by studying several mammalian species, found a positive correlation between the relative medullary thickness (RMT; Sperber 1944) and maximum urine osmolality. These studies have been followed by countless others, exploring the relationship between urine-concentrating ability and RMT in hundreds of species of mammals (e. g. Hewitt 1981; Dunson and Lazell 1982; Lawler and Geluso 1986; Beuchat 1990 b; Beuchat 1996). Other structural indices have been used to show the relationship between the relative size of the renal medulla and the capacity to concentrate urine: ratio of medullary to cortical thickness (M/C; Geluso 1978), percent medullary thickness (PMT; Heisinger and Breitenbach 1969), relative medullary area (RMA; Brownfield and Wunder 1976; Hewitt 1981) and percent medullary area (PMA; Schmid 1972). Nevertheless, the above-mentioned indices must be carefully employed since recent studies on renal morphology have established some limitations in regards to their utilisation. Thus, studies performed by Brooker and Withers (1994), have concluded that for marsupials, only those indices representing relative medullary lenght of the kidneys were correlated to climatic factors, whereas those representing medullary area were not. Moreover, Beuchat (1996) found that the relationship between thickness of the medulla and concentrating ability is neither proportional nor direct.

Sigmodontinae rodents are abundant in many dry areas of South America, but few studies on their physiological adaptations have been published (Mares 1975; 1977 a; 1977 b; 1977 c; Cortés et al. 1988).

Akodon is a polytypic genus that had a rapid adaptive radiation in the earliest Pliocene (5.67 M. y. B. P.; Apfelbaum and Reig 1989) and was distrubuted in South America from humid to semiarid regions with less than 200 mm precipitation per year. Hence, it is an interesting model for studying intrageneric differences in kidney function and structure that could arise as a consequence of selection pressures imposed by environmental constraints.

The aim of this study was to analyse the intrageneric pattern in urine-concentrating capacity and renal morphology of *A. azarae*, *A. cursor*, and *A. iniscatus* that could be the result of adaptive responses to habitats with different mean yearly rainfall.

Material and methods

Experimental animals

Animals of both sexes were collected using Sherman live traps. Thirty four individuals of *Akodon azarae* were captured at Necochea, Buenos Aires Province (38°29′ S, 58°50′ W; rainfall average: 830 mm.year^{-1}; Pampeana biogeographic province; Cabrera and Willink 1973). *A. azarae* is a mouse of moderate size (25 g body mass), strongly associated to natural grassland, particularly to open vegetation formations (Bonaventura 1992; Redford and Eisenberg 1992). This species, which is the more representative rodent species of the pampa's grasslands, is found from southern Brazil to central Argentina (Redford and Eisenberg 1992).

Thirty six individuals of *Akodon iniscatus*, which is a small size mouse (20 g body mass), were captured at Puerto Madryn, Chubut Province (42°77′ S, 65°82′ W; rainfall average 198 mm.year^{-1}; Patagónica biogeographic province). Captured mice were associated with vegetated coastal dunes with scarce availability to water. Distribution of this species also includes the xeric Monte and southern Espinal biogeographic provinces (Cabrera and Willink 1973; Redford and Eisenberg 1992).

Thirty five individuals of *Akodon cursor* were captured at Posadas, Misiones Province (27°22′ S, 55°58′ W; rainfall average 1 604 mm.year^{-1}; Paranense biogeographic province; Cabrera and Willink 1973; Redford and Eisenberg 1992). This species, which is a medium size mouse (40 g body mass), is distributed in southern and central Brazil, Uruguay, Paraguay, and northeastern Argentina. At Misiones province it is found in most habitats but prefers flat and less moist areas (Redford and Eisenberg 1992).

Climatic data records were obtained from the Meteorological Service of the Argentine Air Force. Only adult animals that maintained or gained body weight in the laboratry were used.

Laboratory conditions

Captured mice were carried to the laboratory and housed individually in animal cages (30 × 22 × 15 cm). Wood shavings and cotton for nesting material were placed on cage floors. All animals were maintained under a natural photoperiod (10L : 14D). Temperature ranged from 18 to 25 °C and relative humidity ranged from 50 to 80 %. Animals were fed with dehydrated pellets ad libitum (composition: minimum protein = 21 %; maximum fiber = 4.5 %; minimum fat = 8 %; average calcium = 1.8 %; phosphorous = 1.1 %; maximum ashes = 8 %). Tap water was provided ad libitum.

Salt-water regimen

Tap water and sodium chloride, prepared in distilled water at different concentrations (0.05, 0.15, 0.25, 0.35, 0.45, 1 and 1.5 M), was offered as a drinking source. To measure consumption, a saline solution was provided during 24 h in inverted graduated glass Erlenmeyer, L-shaped drinking tubes, from which animals rapidly learned to drink. An additional inverted Erlenmeyer was placed near the cage to measure evaporation, which proved to be negligible. Once the Erlenmeyer with saline solution was removed, individuals were deprived of food and water and placed in a urine collection apparatus.

Urine collection

Urine was collected in glass vials containing mineral oil by using a collecting apparatus similar to that described by DROZDZ (1975). Urine samples were collected for a total period of 14 h, after which saline solutions were removed and frozen at –20 °C for analysis. Urine samples were discarded when contaminated by fecal material.

Urinalyses

Urine was analysed for total osmolarity measured with an Advanced 3MO osmometer. For evaluating urine-concentration capacity, urine data were analysed as follows: (1) for the whole rank of saline solutions offered (tap water to 1.5 M NaCl) and (2) grouping the data into two categories: low salt concentration (tap water to 0.25 M Na Cl) and high salt concentration (0.35 to 1.5 M NaCl). The latter procedure was performed to mitigate the potential effect of the low number of individuals due to animal supply limitations. ANOVA was used to test the null hypothesis of no effect of saline solution treatments over urine osmolarity. Tukey's test a posteriori was used to identify differences between treatments. T test was used to test the null hypothesis of no effect of saline solution treatments grouped in low or high salt concentration categories over urine osmolarity.

Renal index (PMT)

Data on renal structure (relative size of the renal medulla) was collected as an ancillary measure of urine-concentrating capacity. Within 2 weeks following urine collection, animals were sacrificed by ether inhalation. Fresh kidneys were removed and fixed in 10 % formaline. The kidney tissue was dehydrated and embedded in paraffin wax using standard histological techniques. Serial sagittal sections were cut at 10 µm, and stained with heamatoxylin and eosin. The thicknesses of the cortical and medullary regions were measured to the nearest 0.1 mm with the aid of an ocular micrometer. For each kidney, percent medullary thickness (PMT) was determined by dividing the medullary thickness by the combined thickness of both regions (HEISINGER and BREITENBACH 1969). A mean of 10 measured PMT values was determined for each specimen. ANOVA was used to test the null hypothesis of no differences in relative medullary thickness (PMT) of the kidney among species. Tukey's test a posteriori was used to identify differences between species.

Results

Fluid consumption

Above 0.25 M of saline solution *Akodon iniscatus* and *A. cursor* decreased their fluid consumption as saline concentration increased, whereas in *A. azarae* it was above 0.35 M. Furthermore, *A. iniscatus* drank saline solutions up to 1.5 M, whereas *A. azarae* and *A. cursor* rejected saline solutions above 0.45 M (Fig. 1).

Urinalyses

Akodon iniscatus and *A. azarae* reached a similar urine osmolarity value (≈3 500 mOsmol, ANOVA, $F = 147.9$, $n = 9$, d. f. = 2, Tukey's test, $P > 0.05$). These values were higher

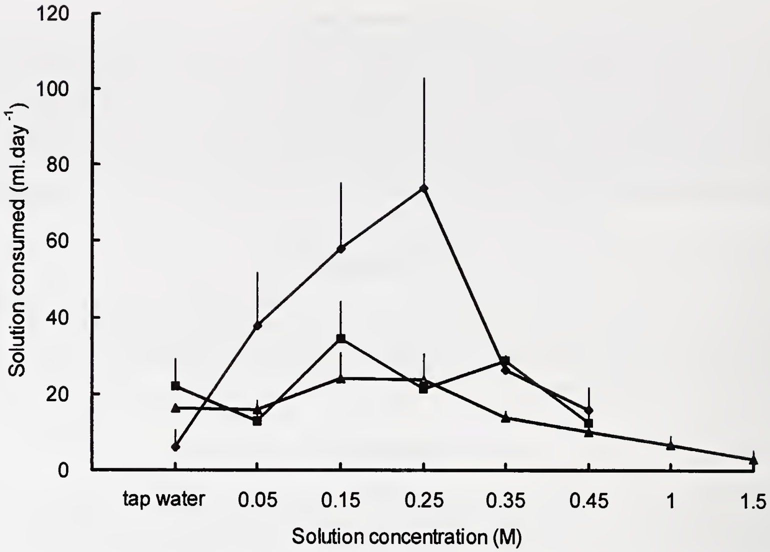

Fig. 1. Fluid consumption of *Akodon azarae* (■), *A. cursor* (◆), and *A. iniscatus* (▲) drinking various concentrations of sodium chloride solution. Vertical lines show +1SD.

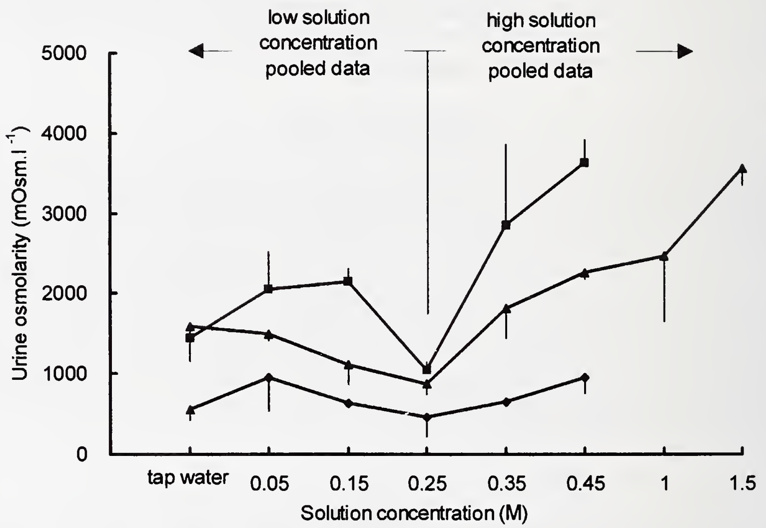

Fig. 2. Total urine concentration for *Akodon azarae* (■), *A. cursor* (◆), and *A. iniscatus* (▲) on fluid regime. Vertical lines show + or – 1SD.

than those observed for *A. cursor* (1 000 mOsmo, Tukey's test, $P < 0.05$). However, *A. iniscatus* reached this value at 1.5 M of ingested solution, whereas *A. azarae* and *A. cursor* reached it at a value of 0.45 M of ingested solution.

The intraspecific comparison of pooled data between low and high solution concentrations (Fig. 2) showed statistical differences in urine osmolarity for both *Akodon azarae* (t-test, n (low) = 12, n (high) = 8, $t = -4.2$, $df = 18$, $P < 0.001$) and *A. iniscatus* (t-test n (low) = 18, n (high) = 7, $t = -5.1$, $df = 23$, $P < 0.001$). On the other hand, these differences were not significant for *A. cursor* (t-test n (low) = 18, n (high) = 7, $t = -1.8$, $df = 23$, $P > 0.089$).

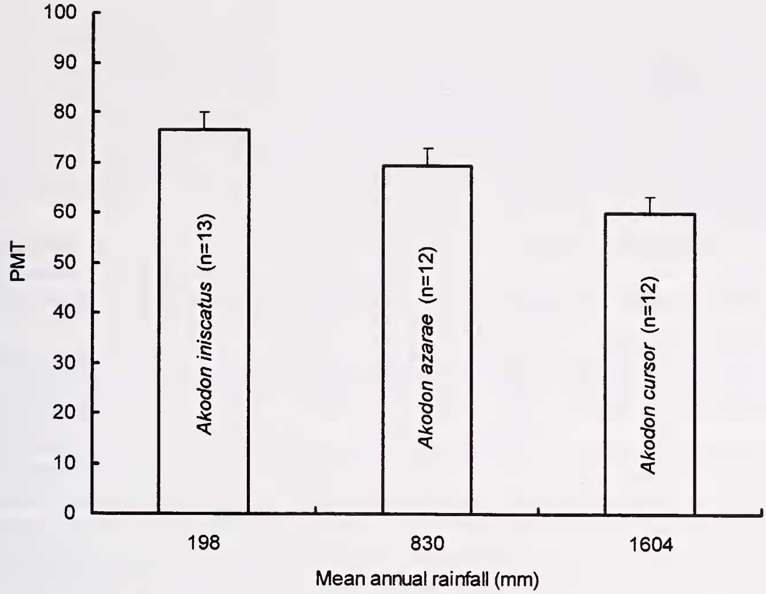

Fig. 3. Comparison of the percentage medullary thickness (PMT) of the kidneys of the selected *Akodon* species from three different rainfall regime areas. Vertical lines show + or – 1SD.

The interspecific comparison of pooled data at low and high solution concentration treatments showed that *Akodon azarae* exhibited the highest urine osmolarity followed by *A. iniscatus* and *A. cursor* (ANOVA, low: $F = 24.74$, $n = 46$, $df = 2$, $p < 0.001$; high: $F = 24.538$, $n = 26$, $df = 2$, $P < 0.03$).

Renal index (PMT)

The three species showed significant differences in percent of medullary thickness (PMT; ANOVA, $F = 71.87$, $n = 37$, $df = 2$; Tukey's test, $P < 0.001$). *Akodon iniscatus* showed the highest mean value (PMT = 76.5 ± 3.4 %, n = 13), *A. azarae* showed the medium mean value (PMT = 69.4 ± 3.6 %, n = 12) and *A. cursor* showed the lowest mean value (PMT = 60.1 ± 3.2 %, n = 12; Fig. 3).

Discussion

Small mammals vary dramatically in their ability to concentrate urine osmotically. Mesic mammals generally have a low capacity to concentrate urine and the rely on drinking as an avenue of water gain as was reported for many families of rodents (Whitford and Conley 1971; Greene and Fertig 1972; Mares 1977 a). On the other hand, small desert mammals cannot rely on drinking and their water requirements must be met by preformed water and/or metabolic water and by reducing the urinary water loss to a minimal level (Brooker and Withers 1994). These adaptations are well documented for *Dipodomys spectabilis* (Schmidt-Nielsen 1964) and *D. merriami* (Nagy and Gruchacz 1994).

Unlike the last rodent species cited above, the three species of *Akodon* studied presently, drink water to survive. However, at high concentrations of water solutions, these species do not drink and a progressive deterioration of body condition was observed.

With respect to urine concentration, urine osmolarity in species of *Akodon* differed after ingesting relatively low or high NaCl drinking solutions. Furthermore, there seems to be different renal handling of excess salt intake among the three species: *A. cursor*, which lives in higher rainfall areas, favor drinking relatively low concentrations of NaCl solution, and choose not to drink high NaCl solutions. In contrast, *A. iniscatus* and *A. azarae*, which live in dryer areas, did not drink substantial amounts of NaCl solutions, but showed good tolerance to high NaCl solutions, by forming a concentrated urine and conserving water.

Therefore, the general pattern of water-salt balance found in the three analysed species of *Akodon* was similar to that reported for *Rattus rattus*, *Phillotis osilae*, *Oryzomys longicaudatus*, *Akodon varius*, and some phillotine rodents (Norman and Baudinette 1969; Dunson and Lazell 1982; Mares 1977 a; 1977 c).

Renal index has been regarded by some researchers as a good indicator of urine-concentrating ability in mammals (Schmidt-Nielsen and O'Dell 1961). Significant relationships between urine concentrating ability, renal index, and the availability of water have been reported for the genera *Sylvilagus*, *Microtus*, and *Peromyscus* (Heisinger and Breitenbach 1969; Heisinger et al. 1973, MacMillen 1983). On the other hand, Beuchat (1993) has found the renal index to be less reliable. Beuchat (1996) also found a significant relationship between the thickness of the inner medulla only in species from mesic environments, reflected in total medullary thickness, and the concentrating ability of the kidney. However, the percent of medullary thickness of the kidney (PMT) for *Akodon* species from xeric, mesic, and humid environments, was related to both the geographic rainfall regime and to its renal-concentrating ability.

Thus, intrageneric differences in urine concentrating capacity and renal morphology would be a consequence of the adaptive radiation of this genus in the earliest Pliocene (5.67 M. y. B. P. Apfelbaum and Reig 1989), related to different selection pressures.

Other influences such as diet, environmental conditions and behaviour affect the overall urine-concentrating ability (Beuchat 1990 a; 1990 b). Studies in natural conditions of these factors would be important to elucidate the physiological adaptations of these species.

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Zusammenfassung

Intragenerische Vergleiche zwischen der Fähigkeit, Urin zu konzentrieren und der Morphologie der Niere bei drei Akodon-Arten aus geographischen Regionen mit unterschiedlichen Niederschlagsmustern

Die Fähigkeiten zur Konzentration des Urins und die Morphologie der Nieren von *Akodon azarae*, *A. iniscatus* und *A. cursor* aus Regionen mit unterschiedlichen Niederschlagsmustern wurden untersucht. Im Gegensatz zu *A. iniscatus* verweigerten *A. azarae* und *A. cursor* NaCl-Lösungen über 0,45 M. *A. azarae* und *A. insicatus* können ihren Urin bis zu etwa gleichhohen Werten konzentrieren. Diese Werte lagen höher als bei *A. cursor*. Die Prozentwerte der Nierenmarklänge zeigen eine Beziehung zwischen dem Niederschlagsmuster der Region und der Urinmolarität.

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